Generation of molecular markers for the identification of isolates of *Puccinia jaceae*, a potential biological control agent of yellow starthistle

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Abstract

Yellow starthistle (*Centaurea solstitialis* L.) (YST) is an important invasive plant that infests millions of hectares in the western USA and Canada. Isolates of the exotic rust fungus *Puccinia jaceae* have been evaluated as a potential biological control for YST. The objectives of this study were to characterize isolates of *P. jaceae* by amplified fragment length polymorphic (AFLP) DNA analysis and to develop primers that will allow the identification and monitoring of isolates. AFLP analysis indicated that Eurasian isolates of *P. jaceae* from different hosts exhibited unique fingerprint patterns primarily based on host/pathogenicity preference. Overall similarities in AFLP profiles of isolates of *P. jaceae* ranged from 68.1 to 94.6%. The three isolates originally collected from YST clustered with similarities of 87.3–94.6%. Simple sequence repeat (SSR) primers were screened for fragments unique to *P. jaceae* pathogens of YST. Two primers, (GACA)$_4$ and (ACG)$_5$, produced 1170 and 912-bp fragments, respectively, in three isolates of *P. jaceae* var. *solstitialis*. The fragments were sequenced, and primers were designed and tested with isolates of *Puccinia* spp. and accessions of *Centaurea* spp. Primers from the GACA repeat amplified a 1091-bp product only with the three isolates of *P. jaceae* that infect YST. Primers from the ACG repeat amplified a 851-bp product with the same three *P. jaceae* var. *solstitialis* isolates, and an isolate collected from *Centaurea maculosa*, which is a weak pathogen of YST. These primers will be useful for monitoring isolates that may be released in future field trials to manage YST populations.
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1. Introduction

Yellow starthistle (*Centaurea solstitialis* L.) is a noxious and allelopathic invasive plant of the family Asteraceae that has successfully colonized millions of hectares of rangeland in the western United States, including California, Oregon, Washington, and Idaho (Maddox et al., 1985; Sun, 1997; Watson and Clement, 1986). Because it is an herbaceous annual (Sun and Ritland, 1998), factors reducing plant health could reduce production of seed and, over time, limit populations. However, yellow starthistle is native to the Mediterranean region (Sun, 1997; Sun and Ritland, 1998) and apparently has few, if

any, natural enemies or pathogens in North America (Maddox et al., 1985). Its establishment and spread is commonly at the expense of native plant species (Sheley et al., 1993; Sun, 1997), especially in disturbed habitats (Maddox et al., 1985), and may be responsible for reduced biodiversity in heavily infested areas.

Biological control of yellow starthistle offers the possibility of population reduction throughout an extensive area with low monetary costs and environmental risks. However, no host-specific North American pathogens of yellow starthistle have been described thus far. The rust pathogen, *Puccinia jaceae* Otth., is found commonly in Mediterranean areas (Savile, 1970a), but has not been reported on yellow starthistle in North America. *Puccinia jaceae* var. *diffusae* was found on diffuse knapweed (*Centaurea diffusa* Lam.) in 1988 in British Columbia,

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Canada (Mortensen et al., 1989), and in 1991 in Wenatchee, Washington (Dugan and Carris, 1992). There have been no reports of this pathogen infecting populations of yellow starthistle in the United States or Canada.

Ongoing research (e.g., Bennett et al., 1991; Bruckart, 1989; Bruckart and Eskandari, 2002; Shishkoff and Bruckart, 1996; Shishkoff and Bruckart, 1993) in a plant pathogen containment facility (Bruckart and Dowler, 1986) has focused on examining the potential of plant pathogens obtained from the native range of yellow starthistle to infect, and ultimately reduce, populations of the host. Isolates of *P. jaceae* collected from different *Centaurea* spp. in Greece, Italy, Turkey, and Yugoslavia varied widely in virulence when inoculated onto yellow starthistle plants (Bruckart, 1989; Shishkoff and Bruckart, 1993). One isolate, 8471 (YST-71), was collected from a diseased yellow starthistle plant in Turkey and was found to be particularly virulent on yellow starthistle (Bruckart, 1989) and worthy of further investigation. While *C. solstitialis* was by far the most susceptible species infected by *P. jaceae* in an extensive study of the range of native and introduced hosts in the tribe Cardueae, limited infection of *Centaurea calcitrapa* L. (purple starthistle), *Centaurea maculosa* Lam. (spotted knapweed), *C. diffusa* (diffuse knapweed), and *Centaurea cyanus* L. was also observed (Bruckart, 1989). In addition, limited infection of *Cirsium pastoris* Howell and *Carthamus tinctorius* L. (safflower) was observed in the host-range risk-assessment used to develop proposals for release of *P. jaceae* for control of *C. solstitialis* (Bruckart, 1989) in the USA. Differences in host-specificity and virulence exist among isolates of *P. jaceae*, but the identification of *P. jaceae* races by morphology is frequently difficult (Savile, 1970a,b). Therefore, it is desirable to examine the genetic diversity among isolates from different hosts and geographic locations, and to have a means of monitoring future field releases.

The objectives of this study were to characterize the genetic variability among *P. jaceae* isolates from different *Centaurea* spp. hosts by analyzing amplified fragment length polymorphic (AFLP) DNA (Vos et al., 1995), and to develop primers that can be used in a simple PCR protocol to detect isolates of *P. jaceae* var. *solstitialis* that will enable monitoring in testing areas infested with yellow starthistle. A preliminary report of a portion of this work has previously been published (Yourman et al., 2000).

2. Materials and methods

2.1. Puccinia isolates

Experiments were conducted in a BSL-3(P) plant pathogen containment greenhouse and laboratory facility at Ft. Detrick, Maryland, under a permit from the USDA Animal and Plant Health Inspection Service. Urediniospores of isolates of *P. jaceae*, and isolates of four other *Puccinia* spp. (Table 1) previously were collected, identified, and inoculated onto hosts from which the isolates had originally been collected, as described previously (Bruckart, 1989). For the AFLP study, urediniospores that were stored in liquid N_2 were allowed to warm to room temperature, inoculated onto host

Table 1
Isolates of *Puccinia* spp. used for AFLP[a] analysis and PCR detection[b] of *P. jaceae* var. *solstitialis*

Isolate No.	*Puccinia* spp.	Disease severity on YST[c]	Plant host[d]	Plant common name	Place of origin[e]
8315[f]	*P. jaceae*	0	*Centaurea diffusa*	Diffuse knapweed	Greece
8409[f]	*P. jaceae*	>20%	*C. solstitialis*	Yellow starthistle	Italy
8462[f]	*P. jaceae*	0	*C. calcitrapa*	Purple starthistle	Turkey
8466[f]	*P. jaceae*	0	*C. calcitrapa*	Purple starthistle	Turkey
8494[f]	*P. jaceae*	>20%	*C. solstitialis*	Yellow starthistle	Greece
85192[f]	*P. jaceae*	<1%	*C. maculosa*	Spotted knapweed	Yugoslavia
8650[f]	*P. jaceae*	0	*C. calcitrapa*	Purple starthistle	Italy
8471[f]	*P. jaceae*	>20%	*C. solstitialis*	Yellow starthistle	Turkey
SAF1[f]	*P. carthami*	0	*Carthamus tinctorius*	Safflower	Mexico
00-226[g]	*P. jaceae*	0	*C. diffusa*	Diffuse knapweed	California
92479	*P. carduorum*	0	*Carduus nutans*	Musk thistle	Maryland
SK-1	*P. chondrillina*	0	*Chondrilla juncea*	Skeletonweed	California
99-001	*P. lagenophorae*	0	*Senecio vulgaris*	Groundsel	England

[a] Amplified fragment length polymorphic (AFLP) DNA.
[b] Detection of *P. jaceae* var. *solstitialis* isolates 8409, 8494, and 8471, with primers based on simple sequence repeat amplification products $(GACA)_4$ and $(ACG)_5$.
[c] Disease severity is based on inoculation of yellow starthistle (YST) with approximately 5×10^4 urediniospores per ml and rated according to the percent leaf area having uredia (pustules).
[d] Plant from which isolate was originally collected.
[e] Country or state from which isolate was originally collected.
[f] Isolate that was used in AFLP analysis.
[g] The only variety of *P. jaceae* identified in North America (see Dugan and Carris, 1992; Mortensen et al., 1989).

plants, and resulting urediniospores were harvested and used for DNA extraction.

2.2. Inoculation of plants with P. jaceae

Seeds were placed into 24-cell seedling trays filled with a soilless mix. Four to five weeks after emergence, plants were spray-inoculated to run-off with an aqueous suspension of approximately 5×10^4 urediniospores per ml. Inoculated plants were placed in a dew-chamber overnight for three successive nights at a temperature of 16–18 °C. During the days, plants were placed in the containment greenhouse. After the third night in the dew-chamber, plants were kept in the greenhouse for both days and nights. The 3-day dew-chamber treatment was repeated 1 week after the first inoculation. Uredinia (pustules) containing urediniospores developed on plants 2 weeks after the first inoculation, and disease severity was determined. Urediniospores on infected plants were collected by a vacuum harvester. Viable urediniospores were maintained in liquid N_2 or at 15 °C for several weeks.

2.3. DNA extraction

For fungal isolates, 1 mg urediniospores per ml of sterile water were placed in petri dishes at ambient temperature overnight. The suspension was placed in 50-ml tubes and centrifuged at 7000 rpm. Spores were ground in a mortar with liquid N_2, and powdered spores were processed with the Nucleon PhytoPure (Amersham Pharmacia Biotech, Piscataway, NJ) extraction kit or the DNeasy Plant Mini Kit (Qiagen, Valencia, CA), following the manufacturer's instructions. DNA was quantified by agarose gel electrophoresis and ethidium bromide staining; an Alpha Imager2000 (Alpha Innotech; San Leandro, CA) densitometer was used to quantify DNA. DNA for yellow starthistle infected with isolate 8471, with pustules covering approximately 10–20% of the leaf area, was similarly extracted.

2.4. AFLP reaction procedures

AFLP primers and adapters were used as described by Vos et al. (1995). Restriction and ligation reactions for AFLP were made with 200 ng of DNA. Procedures included separate restriction and ligation steps followed by preselective and selective PCR amplifications. *Eco*RI adapter pairs (5 μM) (5′-CTCGTAGACTGCGTACC-3′; 5′-AATTGGTACGCAGTCTAC-3′) and *Mse*I adapter pairs (50 μM) (5′-GACGATGAGTCCTGAG-3′; 5′-TACTCAAGGACTCAT-3′) were annealed in a boiling-water bath for 5 min, and allowed to cool to ambient temperature for at least 10 min before adding to

the ligation reaction mix. All reaction mixes were stored at −20 °C.

Preselective primers (*Mse*I + C and *Eco*RI + A) and selective primers (*Mse*I + C and fluorescent-labeled), *Eco*RI + AG-HEX (4,7,2′,4′,5′,7′-hexachloro-6-carboxyfluorescein) or *Eco*RI + AT-FAM (FAM; 6-carboxyfluorescein) were amplified by PCR performed with a PE 9700 thermal cycler (Applied Biosystems, Foster City, CA). For the selective reactions, the concentration of fluorescent-labeled primers of *Eco*RI + 2 bases was increased to 0.3 μM. Internal molecular weight standards of a fluorescent-labeled ROX (6-carboxy-X-rhodamine), 50–500-bp DNA size-standard mixture (Applied Biosystems), were included in all runs.

2.5. AFLP analysis

AFLP-selective amplification reactions were analyzed by capillary electrophoresis with the ABI 310 Prism Genetic Analyzer (Applied Biosystems) with 10 s sample injections and 24 min runs at 15 kV. Amplicons between 50 and 500 bp were analyzed with ABI GeneScan ver. 3.1 software (Applied Biosystems) with the parameter threshold setting of 100 relative fluorescent units (RFU). Amplicon sizes were computed by GeneScan software by interpolation against the ROX size-standard curve. Data tables generated by GeneScan software were analyzed with GelCompar ver. 4.1 (Applied Maths, Kortrijk, Belgium) software to generate binary matrices. Cluster analyses of binary data were conducted by the unweighted pair-group method using arithmetic average (UPGMA) with the software program Phylogenetic Analysis Using Parsimony (PAUP*) ver. 4.0b4a (Sinauer Assoc., Sunderland, MA). Distances were calculated by mean character differences and similarity matrices (Table 2) were calculated by [(1 − mean character difference) × 100] and standardized based on variability of multiple runs of the same isolate. Bootstrap analyses were conducted with 5000 replicates to evaluate the confidence level of cluster patterns. A dendrogram was generated and bootstrap values were labeled at the nodes.

2.6. Design of primers to identify P. jaceae var. solstitialis

Randomly amplified simple sequence repeat (SSR; microsatellite) (Hantula et al., 1996; Zietkiewicz et al., 1994) fragments were generated from isolates of *P. jaceae* (described above) with nondegenerate primers according to Czembor and Arseniuk (1999), except PCR amplification was completed in 20-μl volumes with 4 ng of DNA and 1.5-μM primer concentration. Amplification products were separated with 1.5% agarose gels in 1× TBE (tris-boric acid-EDTA) buffer and stained with ethidium bromide.

Two primers, (GACA)$_4$ and (ACG)$_5$, produced one fragment each that appeared to be unique to isolate

Table 2
Percent similarity of AFLP[a] profiles between isolates[b] of *P. jaceae* and *P. carthami* calculated by UPGMA[c] cluster analysis

Isolate	8466	8315	8409	8494	8471	85192	8462	8650	SAF-1
8466	–	68.1	79.7	74.0	85.9	79.2	89.6	80.1	50.1
8315		–	89.0	85.3	83.0	74.8	72.3	79.6	60.6
8409			–	94.6	94.6	81.5	80.6	76.7	52.2
8494				–	87.3	76.9	73.0	77.9	56.6
8471					–	79.6	82.0	80.2	56.2
85192						–	76.1	75.4	56.4
8462							–	94.2	55.4
8650								–	60.4
SAF-1									–

[a] Amplified fragment length polymorphic (AFLP) DNA.
[b] All isolates were *P. jaceae* except SAF1, an isolate of *P. carthami* (safflower rust) (see Table 1).
[c] Unweighted pair-group method using arithmetic average (UPGMA).

8471, *P. jaceae* var. *solstitialis*. The two fragments were gel-purified (QIAquick Gel Extraction Kit; Qiagen) and used as template for cloning (Topo-TA Kit; Invitrogen, Carlsbad, CA) and sequencing (ABI Prism BigDye Kit; Applied Biosystems). The lengths of the sequences of the two fragments from primers $(GACA)_4$ and $(ACG)_5$ were 1170 and 912 bp, respectively. The sequences were entered into Primer Express software (Applied Biosystems) and primer pairs with complementary annealing temperatures and appropriate amplicon sizes were designed to screen additional isolates of *P. jaceae*.

The primers made to amplify the GACA-derived fragment were: Forward (F), 5′-GGACGGTGAAA GCCTTTCAAG-3′, and Reverse (R), 5′-TCAAGAAA GGACCTAGCACGTG-3′. The primers made to amplify the ACG-derived fragment were: F, 5′-GGT GATCCGATCAAGGTGTTG-3′, and R, 5′-CACGT CCACTTTCGCATCTG-3′. PCR amplifications were completed in 25-μl volumes with 1 μM each of F and R primers and 2 ng of DNA. Thermal cycler conditions were: 94 °C for 2 min, 30 cycles of 94 °C for 20 s, 69 °C (GACA fragment) or 70 °C (ACG fragment) for 30 s, and 72 °C for 1 min. A final extension was completed at 72 °C for 10 min. PCR with internal transcribed spacer (ITS) primers 4 and 5, were included for all isolates as controls ($T_{anneal} = 58\,°C$). Reactions were separated in 1% agarose gels in TBE; 25-μl volumes were loaded into each lane (Figs. 3A–C).

To test the specificity of the primers, DNA template was used from the eight isolates of *P. jaceae*, isolates of *Puccinia lagenophorae* Cooke (groundsel rust), *Puccinia carthami* (Hutzelm.) Cda. (safflower rust), *P. chondrillina* Bubak & Syd. (skeletonweed rust), and *P. carduorum* Jacky (musk thistle rust) (Table 1). In addition, PCR was conducted with DNA from the only known North American isolate of *P. jaceae*, the variety *diffusae* (Dugan and Carris, 1992; Mortensen et al., 1989), which was collected on diffuse knapweed by M. Pitcairn (CA Department of Food and Agriculture) and provided by W. L. Bruckart (isolate 00-226).

3. Results

3.1. AFLP analysis of P. jaceae

A total of eight isolates of *P. jaceae* and one isolate of *P. carthami* (safflower rust) were compared (Table 2) by AFLP and UPGMA analyses combining binary data matrices generated by three labeled primers. Primers *Eco*RI + A, *Eco*RI + AG, and *Eco*RI + AT resulted in 170, 227, and 239 fragments, respectively; a total of 410 fragments were informative. AFLP analysis conducted on eight isolates of *P. jaceae* resulted in unique AFLP fingerprints for each isolate (Fig. 1). Similarities of AFLP patterns between any pair of isolates of *P. jaceae* ranged from 68.1 to 94.6% (Table 2).

Isolates collected from common host plants clustered together (Fig. 2) exhibiting strong bootstrap confidence values. For example, isolates 8462, 8466, and 8650 were collected from *C. calcitrapa* (purple starthistle) (Table 1) and similarities of AFLP profiles ranged from 80.1 to 94.2% (Table 2). These isolates clustered with a bootstrap value of 96%; none of the other isolates were clustered with them. Isolates 8409, 8471, and 8494 were collected originally from *C. solstitialis* in Italy, Turkey, and Greece, respectively, and formed a cluster with similarities ranging from 87.3 to 94.6%, with a bootstrap value of 94%. These three isolates have been identified by morphological characteristics as *P. jaceae* var. *solstitialis* (W. L. Bruckart, USDA-ARS, Ft. Detrick, Maryland, personal communication). Isolate 8315 was collected from *C. diffusa* (diffuse knapweed) in Greece, and joined the isolates 8409, 8471, and 8494 at an internal node, with profile similarity of 89.0, 85.3, and 83.0%, respectively, and a bootstrap value of 97%. Isolate 85192, which was collected from *C. maculosa* (spotted knapweed) in Yugoslavia, had a weak association with the isolates from *C. solstitialis*, but a bootstrap value of only 53%. SAF-1, the *P. carthami* isolate, served as an outgroup and was distinct from the *P. jaceae* isolates. AFLP profile
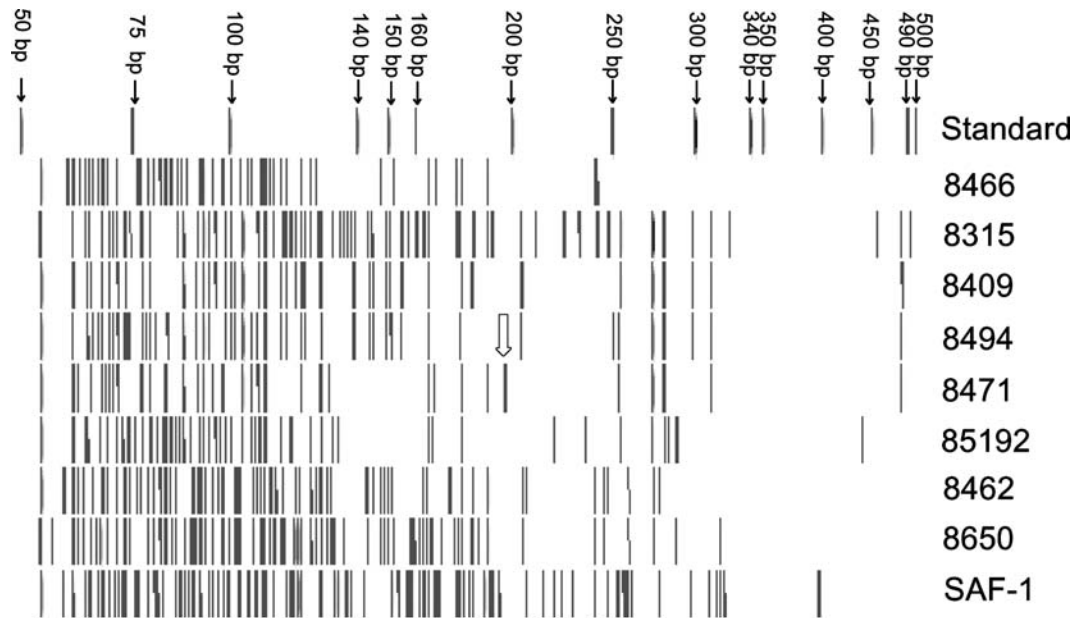
Fig. 1. Gel reconstruction of AFLP profiles generated with DNA extracted from eight isolates of *P. jaceae* (Nos. 8315, 8409, 8462, 8466, 8471, 8494, 85192, and 8650) and one isolate of *P. carthami* (SAF-1). The simulated electrophoresis gel was created with software (GelCompare v. 2.0), using data output compiled from eight individual automated fluorescent capillary electrophoresis runs, using preselective primers *Mse*I + C and *Eco*RI + A, and selective primers *Mse*I + C and fluorescent-labelled *Eco*RI + AG-HEX. Each "lane" represents an individual capillary run. Fine arrows point to base pair (bp) standards indicating DNA standard fragment sizes. The clear arrow in the center (182 bp) points to a marker that appears to be unique to isolate 8471 among the isolates examined.
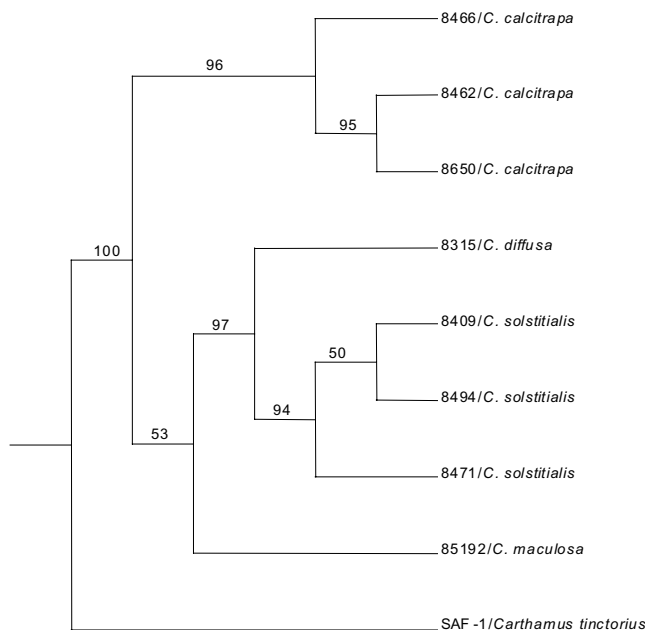

Fig. 2. Dendrogram comparing eight isolates of *P. jaceae* (Nos. 8315, 8409, 8462, 8466, 8471, 8494, 85192, and 8650) and one isolate of *P. carthami* (SAF-1). The species of plant from which the isolates were originally collected follow the isolate number and include, *Centaurea calcitrapa* (purple starthistle), *C. diffusa* (diffuse knapweed), *C. solstitialis* (yellow starthistle), *C. maculosa* (spotted knapweed), and *Carthamus tinctorius* (safflower). Bootstrap values of 5000 replicates are labeled at the nodes. The dendrogram was constructed according to unweighted pair-group method using arithmetic average (UPGMA) distance analysis with the software program PAUP*, v. 4.0b4a (Sinauer, Sunderland, MA).

similarity of SAF-1 to any isolate of *P. jaceae* ranged from 50.1 to 60.6%.

3.2. Detection of P. jaceae var. solstitialis

Two primers, $(GACA)_4$ and $(ACG)_5$, produced one fragment each that appeared to be unique to isolate 8471, *P. jaceae* var. *solstitialis*, in SSR PCR reactions. The unique 1170-bp fragment was cloned and sequenced. The primers designed for fragment-specific amplification produced a 1091-bp amplification product in isolates 8471, 8494, and 8409, three isolates that were originally collected from yellow starthistle (Fig. 3A). These isolates have been tentatively identified as *P. jaceae* var. *solstitialis*. No amplicons were produced from any other isolate of *P. jaceae*, including the North American isolate of *P. jaceae* var. *diffusae* (00-226) (data not shown), or other *Puccinia* spp. In addition, when DNA from the four *Centaurea* spp. was used as a template with these primers, no amplicons were produced. Using ITS primers, amplicons corresponding to the size of the ITS region (∼680 bp) were produced with DNA from all organisms tested, indicating competency of the isolated DNA templates (Fig. 3C). Annealing temperatures of the PCR amplification affected amplification of the 1091-bp GACA fragment. In addition to the amplicons produced from isolates 8471, 8494, and 8409 as described above, a 1091 bp amplicon was produced from isolate 85 192 (a weak pathogen of yellow starthistle, isolated from the closely related *C. maculosa*) when the
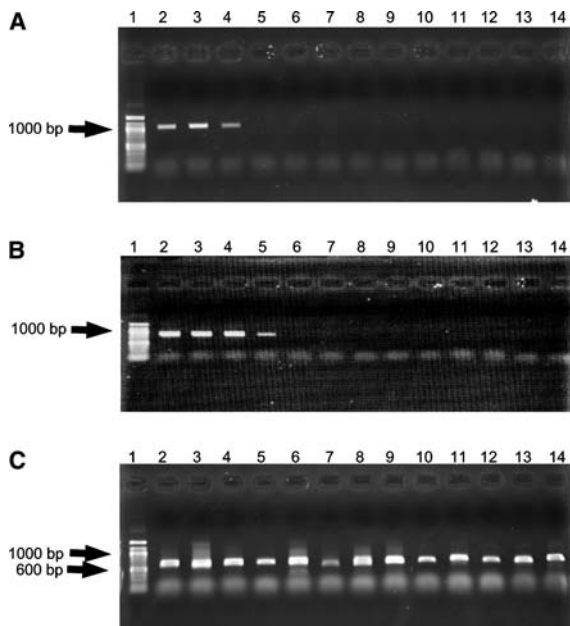
Fig. 3. Agarose gels with amplified fragments produced with primers designed to amplify a portion of randomly amplified simple sequence repeats of (GACA)$_4$ or (ACG)$_5$, originally amplified from DNA of *P. jaceae* isolate 8471, a pathogen of yellow starthistle. The fragments were produced with DNA from isolates of *P. jaceae* var. *solstitialis*, nos. 8471, 8494, and 8409; all are pathogens of yellow starthistle. The additional isolates tested were either weakly pathogenic or not pathogenic to yellow starthistle (see Table 1). Yellow starthistle (YST) DNA was included as a control template. Lanes were: (1) 100 bp ladder, (2) 8471, (3) 8494, (4) 8409, (5) 85 192, (6) 8462, (7) 8466, (8) 8650, (9) 8315, (10) 99-001, (11) SK-1, (12) 92 479, (13) SAF-1. (A) Amplicon fragment length of 1091 bp from primers derived from (GACA)$_4$ repeat. (B) Amplicon fragment length of 851 bp from primers derived from (ACG)$_5$ repeat. (C) Internal transcribed spacer (ITS) regions 1 and 2, and 5.8s of rDNA amplified (∼680 bp) as PCR-positive controls to confirm competency of the DNA template source.

annealing temperature was 67 °C or less (data not shown).

Similar results occurred using the primers derived from the 912-bp fragment amplified with the (ACG)$_5$ SSR primer, except that an additional isolate also produced the amplicon. PCR resulted in an 851-bp amplification product in the three *P. jaceae* var. *solstitialis* isolates 8471, 8494, and 8409 as well as isolate 85 192 (Fig. 3B). Neither of the sequences of the original 1170 (from GACA repeat primers) and 912 bp (from ACG repeat primers) amplification products resulted in homology to known genes when sequences were searched in the National Center for Biotechnology Information (NCBI) Blast database.

DNA isolated from YST leaves infected with *P. jaceae* isolate 8471 did not result in an amplification product with either set of *P. jaceae*-specific primers. Control reactions using primers flanking the entire ITS region, amplified from DNA isolated from infected plants resulted in only a single amplicon corresponding to the size

of the plant ITS region (∼750 bp), rather than the *P. jaceae* (∼680 bp) ITS region (data not shown).

4. Discussion

Biological control has the potential to limit the adverse environmental impact caused when exotic plants invade new areas (Callaway and Aschehoug, 2000; Pimentel, 2000; Strong and Pemberton, 2000). Some isolates of *P. jaceae* appear to be good candidates for biological control of yellow starthistle and related plants such as purple starthistle, diffuse knapweed, and spotted knapweed (Bennett et al., 1991; Mortensen, 1985; Shishkoff and Bruckart, 1996; Watson and Clement, 1986). Although some mild infection of young safflower plants by *P. jaceae* has been reported (Bruckart, 1989; Mortensen et al., 1991; Shishkoff and Bruckart, 1993; Watson and Alkhoury, 1980), the relatively narrow host range of individual isolates of *P. jaceae* (Bruckart, 1989; Shishkoff and Bruckart, 1993) may reduce the ecological problems that have been associated with the release of some foreign organisms for biological control (Pimentel, 2000; Strong and Pemberton, 2000).

The existence of several races of *P. jaceae* that differ in both morphology and host specificity (Savile, 1970a,b; Traquair and Kokko, 1983) makes it important to distinguish isolates that may be successful agents in a yellow starthistle control program. The objectives of this research were to determine the genetic relationship among isolates of *P. jaceae* collected from different *Centaurea* spp. in countries where the pathogen is native, and to develop primers useful for identifying the pathogen in future field releases. Our findings indicated that it might be possible to identify a target isolate among several isolates of the same species. Furthermore, the ability to distinguish isolates with specific host preferences corroborates greenhouse studies by Bruckart (1989) and Shishkoff and Bruckart (1993) that showed distinct differences in pathogenicity among isolates of *P. jaceae* when a range of *Centaurea* spp. were inoculated.

The isolates of *P. jaceae* used in this study were collected in their native Mediterranean range from different *Centaurea* spp. Cluster analysis showed a discrete grouping of isolates with each group correlated to a particular host species. A preliminary molecular phylogenetic analysis of the ribosomal rDNA ITS regions of *C. calcitrapa*, *C. maculosa*, *C. diffusa*, and *C. solstitialis* from seven Mediterranean countries and the USA generated a similar grouping of the four closely related species, suggesting a parallel evolutionary relationship between genotypes of *P. jaceae* and its hosts (Yourman and Luster, unpublished). The country of origin of the *P. jaceae* isolates did not appear to influence genotype similarity. The three isolates originally collected from

C. calcitrapa grouped together and with a significant bootstrap value. The three isolates (8409, 8494, and 8471) that originally were collected from infected yellow starthistle are of the taxonomic subgroup *P. jaceae* var. *solstitialis*. Although the hosts differ, *P. jaceae* var. *solstitialis* and *P. jaceae* var. *diffusae* are similar in spore morphology (Savile, 1970a), and yet the AFLP profiles differentiate the isolates.

The two sets of primers used in this study enabled the detection of the three pathogens available in our collection that are pathogens of yellow starthistle. While the ability to detect an individual isolate may be ideal, because strains or races of *P. jaceae* specific to yellow starthistle have not been identified in North America, these primers should be effective in future monitoring studies.

Although we were not able to detect the pathogen in samples of infected plant tissue, this is likely a technical problem that we are working to overcome with refined extraction procedures. The apparent greater concentration of plant DNA associated with the total DNA complex may have resulted in an overly competitive template environment, which was evidenced by the amplification of the ITS region that was of the size of plant, rather than fungal, ITS.

Results of this research suggest that DNA-based methods, such as AFLP or SSR can be useful in monitoring *P. jaceae* in field trials. Diversity among isolates appears to be large enough to differentiate and identify individual isolates, especially among those of different races (i.e., host preferences). We will continue to optimize the efficiency of the primers and develop new primers that will enable rapid and effective monitoring of field releases of the yellow starthistle pathogen. Ideally the method will entail the sampling and simple processing of infected, possibly presymptomatic, plant tissue to detect and study the movement of the pathogen.

Acknowledgments

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